Investigation of Chemical Essential oil Components of Thymus Kotschyanus in Zagheh Area (In Lorestan Province)

Ali Ariapour[1], Kolsum Karami[2], Amir Heidari Jamshidi[3] and Azita Sadr[4]

[1]Assistant Professor, Department of Natural Resources, College of Range Management, Boroujerd Branch, Islamic Azad University, Boroujerd, Lorestan, Iran(Corresponding author)
[2]M.Sc. Student, Department of Rangeland Management, Young Researchers Club, Boroujerd Branch, Islamic Azad University, Boroujerd, Iran.
[3]Sinior Expert of Cultivation and Crop Breeding, Jihade Agriculture Organization of Lorestan Province, Iran.
[4]M.A. Student, English Language and Literature, Islamic Azad University, Boroujerd Branch, Boroujerd, Lorestan, Iran.

Abstract: From 200 genuses in Lamiacear family there are about 4000 species which one of them is Thymus. Existence of essence in these species is normal that uses for medicinal, nutritional, toiletry and health industry. The genus includes many species in Iran. This study was conducted components of essential oil in biomass of Thymus kotschyanus. Samples were collected in Zagheh area when plant was grown as flowering (in Lorestan Province) in 2011. First of all, anatomical investigations by using coloring and then samples in shadow dried and extracted by Clevenger device as Hydrodistilation method were produced. After producing essences, kind of components and percent of essential chemical components recognized and separated completely by using GC and GC/MS devices. According to components retention volume, retention time, Kovats retention index, mass spectrum and comparing those to standard components the results pointed out that 52 components (about 78.87% of essences in species) as main component such as; Thymol (32.77%), Gamma-terpineol (8.43%), Carvacrol (5.61%), Cynol (4.35%), Borneol (4.35%), Cis-Sabinene hydrate (2.87%), 4-terpineol (2.5%) and Gamma-gurjunene (2.17%). This study and most of the other researches had the same results according to main components of essences in the species but deal was different. It may effected by environmental and husbandry techniques such as; time of collecting, place of plant growing and climatic changes of region factors. These factors effect on biosynthesis of essential in time and place.

Keywords; Thymus kotschyanus, Essential oils, Chemical components, Botanical properties, Thymol

Introduction

The Middle East flora is estimated at 15,000 species. The use of medicinal and aromatic plants, herbs and species in the region has a long history and forms an important part of a number of cultures. Traditional medicine still plays a major role in health care systems despite the availability of modern medicine (Heywood, 1999). The collection, grading and processing of medical and aromatic plants is one of the main income generating activities. The great majority of these plants are still collected from the wild thus endangering the existence of many valuable species. Traditional medicine dates back more than 3,000 years in Iran. Evidence of the use of medicinal plants goes back thousands of years when Avicenna, the well-known Iranian medical scientist and practitioner wrote a volume on medicinal plants upon which western medicine was based until the 13th century (Sabra and Walter, 2000). The flowering plant species of Iran have been estimated to be about 8000 (WHO, 2001). Among 300 to 400 species are used for medicinal purposes. It is one of the largest biodiversity regions in the world containing some of the richest countries in plant resources. Medicinal and aromatic plants constitute the basis of primary health care for the majority of the population in Asia and are a critical source of income for rural populations. The book,

Ghanon in Medicine by Avicenna has been used by the European scientific community for more than 600 years. The works of Avicenna and Razes, another famous scientist have been translated into various languages (Mosaddegh and Naghibi, 2003). Lamiaceae family has about 200 genuses and 4000 species which one of them is Thymus. Majority species of the family have essence and uses for medicinal, nutritional, toiletry and health industry. The genus includes many species in Iran. The medicinal plants are more valuable that since a long time ago are used for treating skin diseases, food, cosmetic, and health industries and pharmacy and its unique properties were demonstrated. The study also included some of the plants used by rural inhabitants as herbal medicines (Amin et al., 2002). Result of Gersbash's (2002), Baran's(2008) and Buyisile's(2009) research show that there is essential oil resource of above of life of Prostanthera ovalifolia, Salvia argentea and Schistostephium heptalobium. Climate and weather are most factors which effect on essence of medicinal plants in each area (Kroger, 2000). Nickavar *et al.* (2005) research on shoot of *T.* daenensis and to get a result most components of the species is Thymol (about 47%). He also in other research in same year resulted that components of *T.* kotschyanus are Thymol (38.6%), Carvacrol (33.9%), Gamma-terpinene (8.2%) and p-Cymin (7.3%). In studies which have been done among 1991 to 1997 by Stahl–biskup, Jemminez and Salgueral the important essential oil in Thymus, Thymol recognized.

The aim of this study was to recognize components of essential oil in biomass of Thymus kotschyanus which collected from Zagheh area of the Lorestan province.

Materials and Methods

Iran with about 1,648,000 km2 areas is located in the southwest of Asia and lies approximately between 25N and 40N in latitude and between 44E and 64E in longitude. Iran's important mountains are Alborz and Zagros. Alborz and Zagros Chains stretch in northwest-southeast and northwest- southeast respectively. The area under study is located in 39º 29′ 52″ N and 48º 24′ 12″ E (Fig. 1). The average annual rainfall of the area is 490 mm, falling mainly in the autumn and winter. Samples were collected in Zagheh area when plants were grown as flowering (in Lorestan Province) in 2011. At the first time investigated anatomical by using coloring and then samples in shadow dried and extracted by Clevenger device as Hydrodistilation method were produced. After producing essences, kind of components and percent of essential chemical components recognized and separated completely by using GC and GC/MS devices. According to components retention volume, retention time, Kovats retention index, mass spectrum and were comparing to those standard components.

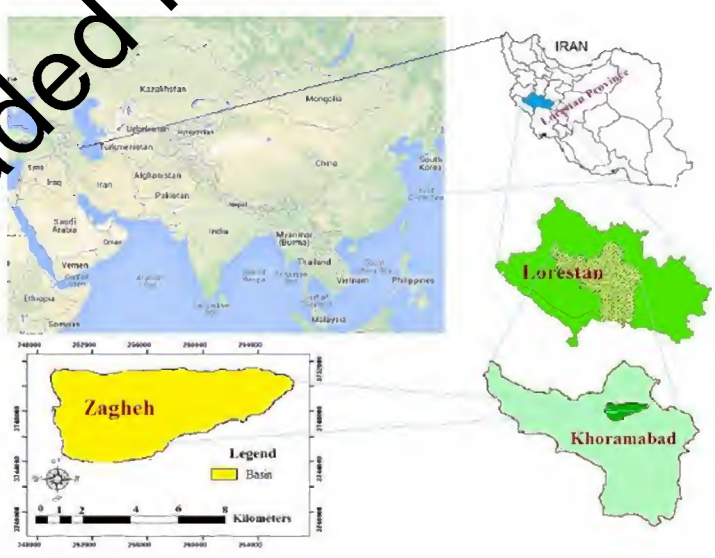

Fig. 1: Location of study area (Iran and Lorestan Province, Zagheh)

Results

Stem of T. kotschanus

Cross cutting prepared of the species show that outer layer is epidermis with non-gland secretory and gland secretory (Fig. 2). Next layer is integument of plant with collanchima and parenchyma. After this layer there is Phloem and Xylem then Marrow in center of integument (Fig. 3 and 4).

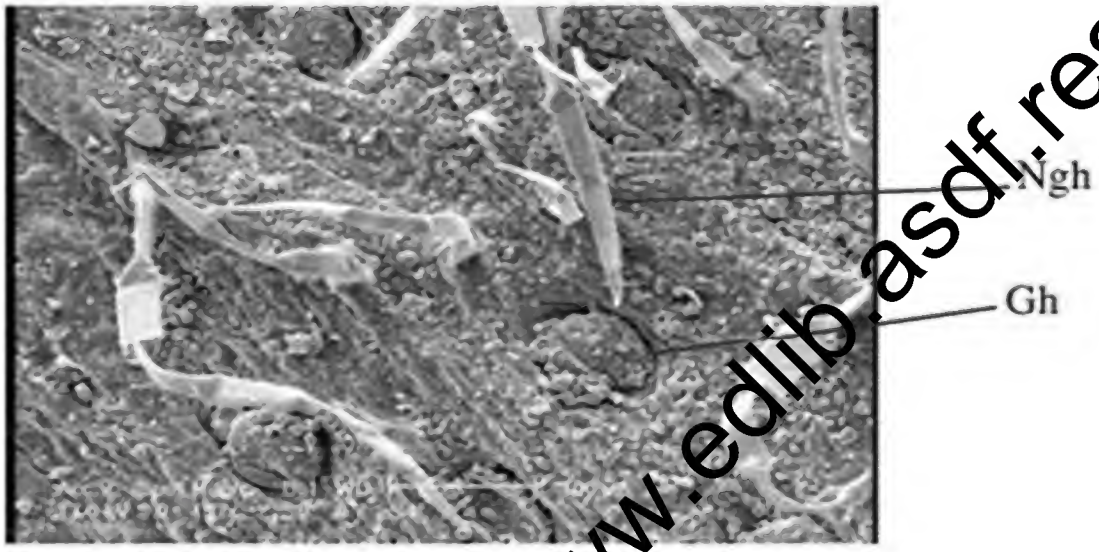

Fig. 2: Stem of the species using by SEM electronic microscope 400X zoom, Ngh: non gland secretory, Gh: gland secretory

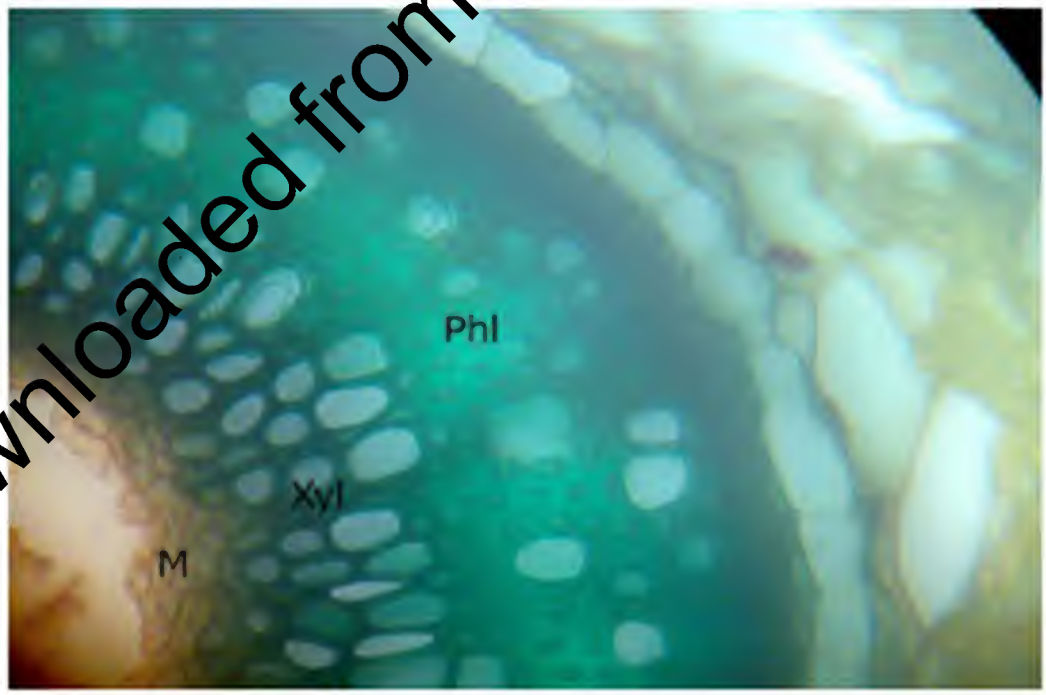

Fig. 3: Cross cutting of Thymus kotschyanus stem with 400X zoom, Ph: Phloem, Xyl: Xylem and M: Marrow

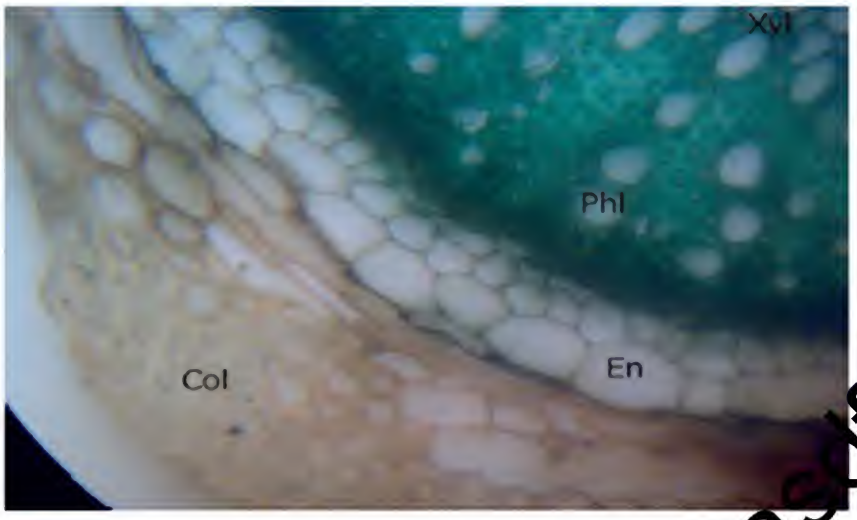

Fig. 4: Cross cutting of Thymus kotschyanus stem with 400X zoom, Ph: Phloem, Xyl: Xylem, Col: Collansim and En: Endoderm

Leaf of T. kotschanus

Microscopic investigations show that leaf of the plant have upper and lower epidermis which in lower epidermis there is fuzz that mainly are non gland secretory and lance-shaped. Fuzzes make by some cells. In the middle there is bunch of Phloem and Xylem (Fig. 5).

Fig. 5: Leaf of *Thymus kotschyanus* using by 400x zoom electronically microscope SEM, Ngh: non gland secretory, Gh: gland secretory

Recognition of components in essence of Thymus kotschyanus in flowering stage

According to components retention volume, retention time, Kovats retention index and mass spectrum and comparing those to standard components results show that there is 52 components in essence that formed 78/87% of all essences. Most of the components in the species were Thymol (32/77%), Gamma-terpinene (8/43%), Carvacrol (5/61%), Borneol (4/35) and Cynol (4/35%) (Tab. 1). Essence gas chromatography spectrum of shoot frequency has been showed in flowering stage (Fig. 6).

Table 1: Recognition components in essence of Thymus kotschyanus in flowering stage

No.	Components	Kovats retention index	Percent	No.	Components	Kovats retention index	Percent
1	Tricyclene	909	0.02	27	4-terpineol	1187	2.50
2	Alpha-thujene	916	0.50	28	Alpha-terpineol	1192	0.25
3	Alpha-pinene	926	0.49	29	Carvacrol methyl ether	1258	0.20
4	comphene	961	0.72	30	Beta-bourbonene	1391	0.06
5	sabinene	970	0.13	31	Beta-elemene	1414	0.10
6	Beta-pinene	967	0.16	32	Trans-caryophyllene	1479	1.67
7	3-octanone	980	0.18	33	Germacrene D	1491	1.25
8	1-octen-3-ol	977	0.11	34	valencene	1492	0.08
9	Beta-myrcene	992	0.66	35	bicyclogermacrene	1360	0.33
10	3-octanol	988	0.04	36	Neryl acetate	1511	0.05
11	Phellandrene	1000	0.10	37	Beta-bisabolene	1470	0.1
12	Delta-3-carene	1006	0.03	38	Beta-cadinene	1526	0.04
13	Alpha-terpinene	1019	1.22	39	Delta-cadinene	1737	0.08
14	Cymol	-	4.35	40	Cis-alpha-bisabolene	1571	1.17
15	1,8-cineole	1009	1.45	41	Geranyl butyrate	1566	0.14
16	limonene	1025	0.17	42	spathulene	1581	0.25
17	Cis-ocimene	1038	0.05	43	Caryophyllene oxide	1582	0.60
18	Beta-ocimene Y	1039	0.71	44	Geranyl peropionate	1539	0.06
19	Gamma-terpinene	1062	8.43	45	Trans-Isolimonene	1469	0.07
20	Cis-sabinene hydrate	1069	2.87	46	Gamma-gurjunene	1469	2.17
21	p-cymenyl	1027	0.02	47	geranic	1285	0.2
22	Alpha-terpinolene	1016	0.19	48	Bornyl acetat	1267	0.18
23	Cis-beta-terpineol	1144	0.38	49	T	1299	32.77
24	Linalool	1098	0.12	50	c ol	1351	5.61
25	comphor	1143	0.06	51	Acetyl thymol	1379	1
26	borneol	1166	4.35	52	Geranyl acetate	1382	0.03

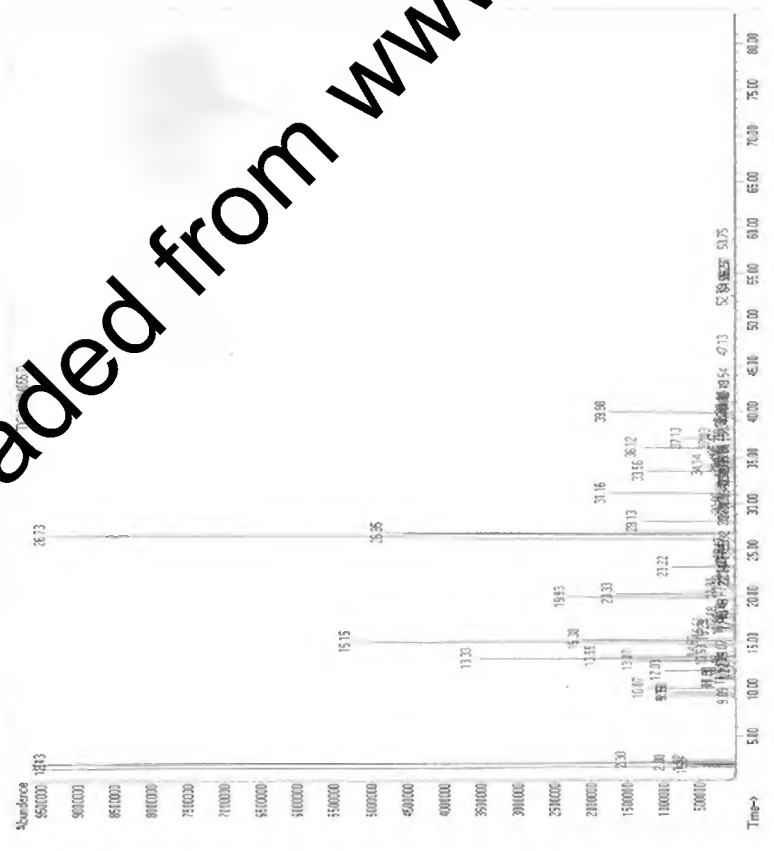

Fig. 6: Essence gas chromatography spectrum of Thumus kotschyanus shoots frequency in flowering stage

Discussion

As researchers in previous studies had been pointed out which weather is one of the most important affecting to essential oil in medicinal plants, this study indicates this fact too. Also weather can change number of components and percent of each components of essence in species, because each spices growth in different environmental factors which cause on number of endocrine glands in lower and upper of leaves. This subject has the same result of Gersbash's (2002), Baran's (2008) and Buyisile's (2009) studies. Also deal of percent of components is different may be because of different niche of the species. It may affected by environmental and husbandry techniques such as; time of collecting, place of plant growing and climatic changes of region factors. This factors effect on biosynthesis of essential in time and place.

Resources

1. Adams, R.P., 2004. Identification of Essential Oil Components by Gas. Chromatogeraphy/Quadrupole Mass Spectroscopy. Allured Publishing Corporation, Carol Stream, 456p.
2. Amin, G., Dehmoobed-Sharifabadi, A., Surmaghi, M. H. S., Yasa, N., Kyrechi, Y., Emami, M., Shidfar, M., Amin, M., Moghadami, M., Kordbacheh, P. and Zeini, F., 2002, Screening of Iranian plants for antifungal activity: Part I. Available at: http://www1.tums.ac.ir/daru/DaruVolum10-No1-2002/Amin1.htm (31 Jul. 2003)
3. Baran, P., & Ozdemir, C., & Aktas, K., 2008. Morphological and anatomical properties of *salvia argenteal*, Research journal of agriculture and biological sciences, 4(6), 725-733.
4. Buyisile, M., 2009, Morphological and Chemical Composition of the essential oil of the leaf of *schistephium heptalobium*. African journal of biotechnology, 8(8), 1509-1519.
5. Cowan, M. M., 1999. Plant Products as anti microbial agents, Clinical microbiology Reviews, 12, 564-582.
6. Davies, N. W., 1990. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and carbowax 20M phases, Journal of chromatography, 503(1): 1-24.
7. Gersbash, P.V., 2002. The essential oil secretory structures of *prostanthera ovalfolia* (Lamiaceae), Annals of Botany, 89, 255-260.
8. Harborne, IB., 1998. Introduction to ecological biochemistry, 3rd edition London, Academic-press.
9. Jemminez, J., Navarro, M.C., Monfella, N.P., Martin, A., Martinez, A., 1993. *Thymus zigis* oil: Effect on cc14-indoost capatotoxicity and free radical scan venjer activity. J., Esset. Oil Res, 5, 153-158.
10. Heywood, V., 1999, Plant resources and their diversity in the Near East, In: International Expert Meeting on Medicinal, Culinary and Aromatic Plants in the Near East, Forest Products Division FAO Forestry Department and the FAO Regional Office for the Near East, Cairo, Egypt, 19-21 May 1997.
11. Karaman. S., Digrak. M., Ravid. U., Ilcim. A., 2000. Antibacterial and antifungal activity of the essential oil of *Thymus revolutus* Celak from Turkey. Journal of Ethnopharmacology 76(2001) 183-188.
12. Maria, C., Rota., Antonio, H., Rosa, M.M., Jose A.S., Maria, J.J., 2008. antimicrobial activity and chemical composition of *Thymus Vulgaris, Thymus Zygis and Thymus hymalis* essential oils, Food control 19, 681-687.
13. Miguel, G., Simoes, M., Figueiredo, A.C., Barroso, J.G., Pedro, L.G., Carvalho, L., 2004. Composition and antioxidant activities of the essential oils of *Thymus mastichina*, Food chemistry 86, 183-188.
14. Mosaddegh, M. and Naghibi, F., 2003, Iranian traditional medicine, past and present. Available at: http://www. islamset.com/ioms/cam/Abstracts/mosadegh.html (30 Jul. 2003).
15. Sabra, A. and Walter, S., 2000, In: (Eds.), Country reports, In: Non-Wood Forest Products in the Near East: A Regional and National Overview, Non-Wood Forest Products Programme FAO, Rome, Italy.

16. Nejad Ebrahimi, S., Hadian, J., Mir Jalili, M.H., Sonboli, A., Yousefzadi, M., 2008. Essential oil composition and antibacterial activity of *Thymus caramanicus* at different phonological stages, Food chemistry 110(2008) 927-931.
17. Nickavar, B., Mojab, F., Dolat-Abadi, R., 2005. Analysis of the essential oils of two *Thymus species* from Iran. Food chemistry 90(2005) 609-611.
18. safaei-ghomi, j., ebrahimabadi,h., Djafari-Bidgoli, A., Batooli, Z., 2009. Gc/ms analysis and invitro antioxidant activity of essential oil and methanol extracts of *Thymus caramanicus* jalas and its main constituent carvacrol, Food chemistry 115(2009) 1524-1528.
19. Salgueral, L.R., Vila, R., Tomi, F., Figueiredo, A.P., Barroso, J.G., Canigueral, S., Casanova, J., Cunha, A.P., Azadet, J., 1997. Competition and variability of the essential oil of *Thymus caespitius*, From, Portugal photochemistry 45,307-11.
20. Stahl–biskup, E., 1991. The chemical composition of Thymus oil, J. Essent, Oil. res. 3(61-82).
21. WHO, 2001, Legal Status of Traditional Medicine and Complementary/Alternative Medicine: A Worldwide Review, WHO/EDM/TRM/2001.2, WHO, Geneva, p. 188.